November 18, 1951.

Dear Ed:

To answer some of the points raised in your letter of the 13th:

The effaciency of replica transfer varies according to many conditions; it is probably relatively higher when there are relatively few cells. Clones of 50 or more cells probably have annexcellent chance of being carried over. From an initial plate of nutrient agar, with 100 auxotrophs and 100,000 prototrophs, incubated a few hours, almost all of auxotrophs were carried over to the final plate of the series N.A. -velvet- Minimal agar- velvet- sm agar (to characterize the auxotrophs used). This should not be a major difficulty, and it would not matter much if the efficiency were not perfect unless the mutant clones were very small compared to the prototroph.

Whether 4x as many plates is better or worse than repeated pourings and digging out auxotroph colonies is a matter of taste. I hope I can be excused for saying that I never did like delayed enrichment.

It looks to me as if you have an interesting mutant, i.e., one which can be propagated indefinitely from large inocula on minimal medium. Have you studi it in liquid medium? I don't know how to avoid the carryover, except possibl by using less fully grown initial plates. Quite small colonies give satisfac- tory replicas, or, you could "dilute" out the cells by a first replica, makin the second immediately afterwards.

My student finished his practice experiments to try to work out the penicill repeated replicas. He obtained only a limited enrichment of auxotroph clones (contra my remarks in a letter to Mike a couple of days ago). The probl is to work out how long and at what concentration of penicillin in minimal a to expose the replicated clones. I strongly suspect this could be done, but hope you do it. Lately, we have preferred to obtain our mutants (for genetic work) without using any mutagen, by penicillin selection in liquid medium, an subsequent screening with replica plating.

Sincerely,

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